

**“MARKED UP COPY TO SHOW CHANGES”**

On page 30, first paragraph:

The two halves are then placed together in a PCR mix along with the TCRBV- and TCRBC-specific primers only and amplified in a 'hot-started' PCR reaction. During this second round of amplification, the 2 halves are annealed together by virtue of their overlapping mutation sequence and a new mutant template is created by the PCR process. The new PCR product can then be re-cloned back into a vector such as PCRscript and sequenced until an error-free clone is identified. Using this process we have replaced the SEQ ID NO: 1 (CATCAGAAGCAGAGATCTCC) sequence in the wild-type TCRBC region with the SEQ ID NO: 2 (GATGTCAAGCTGGTCGAGAA) sequence from the corresponding region of the TCRAC gene. This mutation was designed not to affect the overall size, dG/dC:dA/dT content or the primer annealing sequences of the original wild-type template. The mutated template amplifies with equal efficiency as the wild type template in a number of TCRBV-specific PCR reactions. See Higuchi R., et al (1988) *Nucleic Acids Res.* 15, 7351-7367 and Ho S.N., et al (1989) *Gene* 77, 51-9, for further details on gene SOEing.

On page 36 first paragraph:

Alternatively, as the TCRBJ segments BJ1S1, BJ2S1 and BJ2S7 seem to be the most frequently used, measuring the TCRBV-BJ combinations for just these three segments will approximate to 40-45% of the total V $\beta$ -specific T cell gene rearrangements for most V $\beta$ s [Jeddi-Tehrani *et al* (1994) *Human Immunology* 40, 93-100]. Quantitation of TCRA mRNA, total and specific, may also be achieved using the RT-CPCR method. In the first instance, TCRA mutants have to be manufactured. In the reverse of the TCRB method, mutants have the SEQ ID NO: 2 (GATGTCAAGCTGGTCGAGAA) wild-type TCRAC sequence replaced with the equivalent sequence from the TCRBC chain, SEQ ID NO: 1 (CATCAGAAGCAGAGATCTCC) by gene SOEing. This may be performed to sequences derived using TCRAV- and TCRAC-specific primers (for quantification of specific  $\alpha$  chain message) or in sequences amplified using two TCRAC-specific primers (for quantitation of total  $\alpha\beta$  mRNA).